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STUDIES OF THE DEVELOPMENT OF THE PIPERACEAE

II. THE STRUCTURE AND SEED-DEVELOPMENT OF *PEPEROMIA* HISPIDULA

(Continued from page 339 of this volume)

DUNCAN S. JOHNSON

D. THE CARPEL AND FRUIT; THE OVULE AND SEED

The development of the ovary of this *Peperomia* is initiated by a swelling out of the base of the floral bract, due to the elongation of the subepidermal cells of the upper side of its stalk (figs. 24, 25, 26). This elevation, convex at first, later becomes flat, and then concave, as the rapidly growing margin pushes upward to form the wall of the single celled ovary (figs. 53, 54). Before this wall closes together to form the stigma, the floor of the ovarian cavity has begun to swell upward to form the single erect ovule, in the midst of which the primary archesporial cell has now become evident (figs. 54, 55). The carpellary tube closes in to form the stylar canal at about the time the parietal cell is separated from the embryo-sac mother-cell (fig. 56). It is noteworthy that, from early in its development, the abaxial margin of the upgrowing carpel is longer than that next the axis (figs. 55, 56). This longer side ultimately gives rise, at its tip, to the papillose, rounded, simple stigma (figs. 56, 57, 78).

The wall of the carpel is at first only three or four cells in thickness (fig. 55). The portion about the ovule increases but little in thickness thereafter, and in the mature fruit is only 4 or 5 cells thick, except in one longitudinal strip along the abaxial side. There, in the mature fruit, it may become 6 or 7 cells in thickness (figs. 78, 100, 101, 108). In the stylar region, however, periclinal walls appear more abundantly, especially on the abaxial side, and a section of the style may show it to be 16 or 18 cells in diameter (figs. 78, 79). Of the 4 to 7 layers of cells

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making up the wall of the ovary, only the innermost and outermost are appreciably specialized in structure. The only exception to this is the single delicate strand of vascular tissue running down the abaxial side (figs. 100, 101, 108). This strand is well-developed by the time the embryo sac is mature (fig. 78). It consists from this time on of but 2 or 3 ringed ducts, 3 or 4 μ in diameter, and of a few small, elongated cells nearby, that may play the part of phloem (figs. 100, 101, 108.) This simple vascular bundle connects with the lattice-like tracheids of the style above, and with the vascular bundle of the stalk of the fruit below. Aside from these vascular tissues, the 2 to 5 cell-layers of the carpel between its inner and outer layers, consist of but slightly elongated, thin-walled parenchymatous cells (figs. 101, 108).

Up to a late stage in the development of the fruit, the cells forming the innermost layer of the carpel remain thin-walled, and each may contain two or three dozen chloroplasts. In the mature fruit, however, the inner walls of these cells and the inner portions of all four radial walls become slightly thickened, and the whole cell contents turn brown and shrink against the inner walls of the cell (fig. 108). The chloroplasts of these cells, though shrunk and brown, do not lose their identity, as they do in the dried contents of these same cells in *Peperomia pellucida* (Johnson, 1900a, fig. 15). Neither are the walls of these cells thickened to the same degree, nor in the complex lattice-like pattern of those of *P. pellucida*.

The outer epidermal layer of the carpel is far more highly specialized than any other. It consists, when mature, of four distinct types of cells, the oil-cells, the hydathodes, the bristle-bearing cells and the ordinary less modified epidermal ones. The latter differ in size and shape (figs. 107, 108). Their convex outer walls are slightly thickened with a corrugated cuticle, like that of the epidermis of the stem and leaf (cf. fig. 13). The oil-cells are structurally the least modified of the other surface cells of the fruit. They are nearly isodimensional, and are commonly shaped like truncated pyramids with their bases inward (figs. 78, 100, 107, 108a). Up to the time of the maturing of the embryo sac in an ovule, the protoplast in each of the oil-cells of its carpel is thick and the nucleus and vacuole about half the diameter of the cell. In the mature fruit the protoplast is usually somewhat shrunk and, in unstained material, only a few small yellowish granules are visible. Imbedded in the part of the protoplast lying against the outer wall, however, is a disk of a substance that stains a dense black with iron haematoxylin (figs. 100, 108, 108a).

The glandular hairs, or hydathodes, as they probably are in function, occur only on the stalk, style, and adjoining base and tip of the ovary (figs. 78, 100, 108). The structure of these hydathodes is the same as that of those occurring on the leaf. With the ripening of the fruit the hydathodes may shrivel somewhat and turn brown, and the outer cells may sometimes drop off (fig. 108).

The most highly specialized of the cells of the surface of the fruit are the tapering, unicellular bristles. These project radially from all sides of the swollen fertile portion of the ovary, and are found nowhere else on the plant (fig. 107). Each bristle is initiated by the protuberance of one of the larger surface cells of the carpel, which occurs at about the time the endosperm nucleus is being formed by fusion (fig. 101). The cell continues to elongate until it attains a long conical form with a diameter of 8 or 10 μ at the base, and a length of 300 or 400 μ . The mature bristle has from 2 to 6 longitudinal ribs along the inner surface of its wall, each of which may become 2 or 3 μ in thickness and 5 μ wide (figs. 108, 109). At the base of the bristle these ribs run down to the inner wall of the epidermal cells, and there may be bent outward, thus bracing the bristle more effectually (fig. 108). These bristles remain intact, stiff and rigid, on all the mature fruits seen. It is therefore, possible that they may be effective in keeping snails and small insects off the fruits, but no conclusive evidence has been obtained on this point. It is of interest to note the relatively late appearance of these bristles, as compared with that of the multicellular hairs of the leaf, which appear while the latter is still very young.

Besides the middle, fertile portion of the ovary, with which we have thus far been concerned, there are certain interesting structures to be noted in the basal stalk and the terminal style and stigma. The stalk of the ovary and of the fruit, though not as long as in species like *P. obtusifolia*, is still quite distinct (figs. 78, 100, 107). It has a length, from the chalaza to its insertion on the axis, of about half a millimeter. Its diameter is about .2 of a millimeter. The epidermis of the stalk is like that of the body of the fruit, except that bristles are absent and hydathodes are abundant. The most interesting feature of the internal structure of the stalk is the vascular tissue. For two thirds the way downward from the chalaza there are two distinct groups of these cells. One group is central or axial; the other, coming down from the abaxial side of the body of the carpel, does not fuse

with the proper bundle of the stalk until the base of the stalk is nearly reached (figs. 100, 108). The central vascular strand consists of but 3 or 4 xylem elements, and perhaps a few phloem elements. These are embedded in a mass of parenchyma. The central bundle ends above, with a considerable number of bent and spiral tracheids, just below the thickened cells of the chalaza (fig. 108). The walls of these latter cells begin to thicken before the fruit has attained its mature size. It seems evident that food material brought up by the bundle and the parenchyma of the stalk must be able to pass through these partially thickened cells of the chalaza, for the walls of the integument, the only other possible path to the ovule from without, are at this time, still more greatly thickened. The stalk, as has been stated above, finally ruptures at the constricted portion of the base, and thus sets the ripe fruit free. At this constriction the stalk is abruptly narrowed to half the diameter, it has immediately above, and the epidermal cells are very thin-walled. Both these features of the structure make the break easier, and strictly localize it (figs. 100, 107, 108).

The style and stigma are developed by the continued growth of the upper margin of the carpellary ring, after the latter has closed together above the ovule to form the distinct stylar canal (figs. 56, 57). The stigma is formed solely from the abaxial and longer lip of this upgrowing carpel (figs. 78, 100). It consists, when ready for pollination, of a knob or rounded end made up of parenchyma, and covered by a layer of papilla-tipped columnar cells. These latter have large nuclei and dense cytoplasts, and form the surface for the attachment of pollen grains (fig. 78). With the initiation of the endosperm the mature stigma shrivels and remains on the ripe fruit as a cap of black cells at the end of the style (fig. 107). No germinated pollen grains were detected on the stigma.

The style of this plant which is remarkably long for a *Peperomia*, is marked off from the stigma by a definite constriction (fig. 78). From this point down to the shoulder of the fruit the style is 180 to 200 μ long and about 150 μ in diameter. The ordinary epidermal cells are interspersed with oil cells and hydathodes, but no bristles (figs. 100, 107, 108). Internally the style consists of more or less elongated cells, which are at first much alike (fig. 57). Later longitudinal walls, in the neighborhood of the stylar canal, give rise there to a small-celled tissue, with dense protoplasts, which evidently serves

as a conducting tissue for the pollen tubes (figs. 78, 100). At least, the only structures seen that resembled pollen tubes were growing through this tissue. Latticed tracheids, to the number of 10 or 12, and with a diameter of from 10 to 25 μ , arise in the tissue of the abaxial side of the style. These tracheids form the expanded upper end of the slender vascular strand that runs along this side of the fruit (figs. 78, 100, 107). The position and structure of these tracheids suggests that they may serve for water storage, perhaps to prevent premature shriveling of the style and stigma. These cells, however, do not reach their highest development till some time after pollination.

We may now take up the details of development of the ovule, with its single integument, into the seed with its seed coat.

The nucellus of the ovule is initiated by the upward stretching of the subepidermal cells at the center of the carpellary ring (figs. 53, 54). The mound so formed continues its growth until, by the time the carpel has closed together above, it forms a cone of about 30 cells in vertical section (fig. 56). The growth of the nucellus continues until, by the time the egg is differentiated, it is 10 to 15 cells broad and 25 to 30 cells long (fig. 78). The cells of the peripheral layer are somewhat flattened radially, while in the interior of the ovule the short diameter of the cells at this stage is the longitudinal one. The group of cells above the sac, formed by the division of the parietal cell, consists at this stage of 8 or 10 cells that show in a longitudinal section (figs. 81, 89). In ovules that do not give rise to embryo and endosperm, growth of the nucellus stops at the stage just indicated. Many ovules that have reached this stage and then shrivelled up are found on spikes bearing ripe, or nearly ripe fruits. This shriveled condition is probably due to the lack of fertilization of the eggs in these ovules. It is true that fruits are found in which degeneration set in at an earlier, or at a later stage of development, but in by far the larger number of the ovules that degenerate, this process seems to begin after the embryo sac is mature and ready for fertilization.

In those seeds where embryo and endosperm are formed, a very marked renewal of growth of the nucellus occurs. This growth is due only in small part to increase in the number of cells present at the beginning of embryo-formation, but chiefly to an increase in the size of these cells (figs. 78, 100, 108, 111). The nucellus increases from 125 to 900 μ in length, and from 90 to 590 μ in diameter. The highly vacuolated cells seen in the nucellus in the earlier stage become the

starch-filled perisperm cells of the ripe seed (figs. 101, 108, 111). The starch grains of the perisperm cells are, as in the seeds of *P. pellucida* and other species, grouped in the several distinct vacuoles of each cell, to the number of often 1,200 or 1,500 in a single vacuole. The nucleus of the ripe perisperm cell is usually squeezed up in a small central or lateral portion of the cytoplasm between the vacuoles. There is sometimes present also a large rounded body, staining slightly, which, like that found in *Peperomia pellucida* (Johnson 1900a, fig. 15), is probably to be regarded as a leucoplast. The cells of the outer layer of the nucellus, including those above the embryo sac, have much less starch than the central cells. In the ripe seed the starch has entirely disappeared from several layers of the cells next the embryo sac. The walls of the cells nearest the sac have completely collapsed (figs. 89, 108). This perisperm, with its starch, is absorbed through the endosperm at the time of germination, as in *P. pellucida*, which will be shown later (fig. 111).

The integument is initiated soon after the carpel closes in above the ovule, chiefly by the activity of the epidermal cells of the base of the young ovule (figs. 56, 57). This ring-like outgrowth continues pushing upward as the nucellus elongates and closes together above to form the micropyle, before the time when the embryo sac has reached the sixteen nucleate stage (figs. 74, 78). The integument is two-layered at first, and most of it remains so (figs. 68, 78, 100). At the base of the ovule and occasionally near the micropyle, it may become three-layered as the fruit approaches maturity (fig. 108). The cells of the outer layer of the integument never thicken appreciably on the sides of the fruit, but collapse and are finally crushed between the inner layer of the integument and the inner layer of the carpel (figs. 101, 108). At the base and the apex of the ovule one or both of the outer layers of the integument may be thickened like the inner one (figs. 100, 108). This inner layer consists of cells which are thin radially and isodimensional in the other two directions (figs. 100, 101). The nuclei remain large and active until a late stage of development. The inner and outer walls attain a thickness of $.5\ \mu$ or $.7\ \mu$, and have a dark brownish color in unstained material. Near the micropyle the inner walls of these cells are thrown into strong folds and protuberances, giving them a very irregular outline in section (figs. 89, 100, 108, cf. Johnson 1900a, fig. 15; 1910, fig. 68). The nuclei of these cells are large and active at first, but later these and the other cell contents

become distorted and shriveled. This layer is clearly the chief protective layer of the seed. Near the base of the ovule the cells of the two or three layers of the integument may become thickened and brown (fig. 108). Across the base of the nucellus a series of 5 or 6 layers of transversely expanded cells become thickened, and thus serve as a protection against desiccation of the seed on this side (figs. 100, 108). The walls of these cells become brown, but are not as greatly thickened as those of the integument itself.

It is clear that taken all together the structure of the carpel and seed coat of this *Peperomia* is much less highly specialized as a protection against desiccation than that of these same structures in *Peperomia pellucida*. In fact all other species of *Peperomia* examined by the writer show greater thickening of these tissues than is seen in *Peperomia hispidula*. It therefore seems altogether probable that the present character of these structures in the latter species has arisen secondarily, and is related to the character of its habitat.

The vascular system of the carpel, with its one slender bundle, is possibly to be regarded as secondarily derived from types like the *Pipers*, with six longitudinal vascular bundles in the carpel (Johnson, 1902, fig. 15). It must be recalled that the development of the ovary of *Peperomia hispidula* gives no hint that it has arisen by the coalescence of three carpels, which does seem true of most species of *Piper* that have been studied.

E. THE EMBRYO SAC, EMBRYO AND ENDOSPERM

1. *The archesporium and tapetum.*

Of the group of hypodermal cells visible in the young ovule from the beginning, one axial cell becomes distinguishable, about the time the carpel is closing together, by its larger size and larger nucleus. This cell divides at about the time the integuments are initiated, to form a parietal or tapetal cell above and the definitive archesporial cell toward the chalaza (figs. 56, 57). In a few ovules two such archesporial cells were seen (figs. 113, 114), which as we shall see may often go on in their development and form two embryo sacs (fig. 120). No mitoses of the nucleus of the primary archesporial cell were seen, but from mitoses occurring just before and after this stage there can be no doubt that this mitosis is a normal vegetative one in which more than 20 chromosomes are concerned.

The tapetal or parietal cell early divides by 1 or 2 periclinal and two or three series of anticlinal walls to form the 2 or 3 layers of parietal tissue found in the young seed above the sac (figs. 74, 77, 81, 89; also Johnson, 1907, fig. 2). As the seed ripens the walls of these cells become but slightly thickened. The cells of the layer next the integument often divide by a few more anticlinal walls and elongate radially to form a series of columnar cells just below the micropyle (fig. 104). In the ripe seed the layer of parietal cells next the embryo has been crushed by its growth, the remaining layers persist intact and plump, but contrast with the perisperm cells in having few starch grains in them. At germination these parietal cells are pushed off, along with the style of the fruit, by the swelling of the endosperm as it bursts the seedcoats (see p. 375; fig. 111).

The definitive archesporial cell, or embryo sac mother-cell, is at the time of its formation nearly cubical in shape. It is about $20\ \mu$ long and $16\ \mu$ wide. Its nucleus when first organized is about $10\ \mu$ in diameter, has a distinct wall, a prominent nucleolus and a rather close chromatin net just within the wall (fig. 58). As development proceeds this embryo sac mother-cell grows, until, when ready for the next nuclear division, it may be $30\text{--}45\ \mu$ long and $25\text{--}30\ \mu$ broad. The nucleus itself continues to grow, up to and even after the time of synapsis. During the early phases of synapsis the nucleolus may have a diameter of 3 or $4\ \mu$. Just before synapsis sets in the chromatin of the nucleus lies near the periphery in the form of a net, the threads of which have a diameter of $.3$ or $.4\ \mu$ and form meshes 1 to $3\ \mu$ across (figs. 58, 59). The cytoplasm up to this time retains a uniform structure, with rather evenly distributed small vacuoles.

2. *The megaspore and embryo sac.*

The development of the embryo sac from the definitive archesporial cell is initiated by the occurrence of a genuine synapsis. The first evidences of the beginning of this process are seen in the increase in thickness of the chromatin threads of the reticulum, in the reduction in the number of meshes and in the gradual shrinking away of the chromatin from most of the nuclear wall. Later the whole chromatin thread forms but 1 or 2 close tangles of contracted loops near one side of the nuclear cavity (figs. 59, 60, 61). The examples of this stage seen do not give evidence of a doubling or splitting of the chromatin thread, before the compact knot stage is reached (figs. 60, 61). On emerging from the characteristic tight knot the chromatin takes the

form of a rather loosely looped spireme, with distinct chromomeres scattered along it (fig. 62). These chromomeres are about half a μ in diameter and occur at intervals of 1.5 or 2 μ along the thread. The intervening portions of the thread are slightly narrower and stain much less deeply. The thread at this stage is evidently double (figs. 62, 120-19. 2-30. 6). Soon after this spireme appears it begins to segment, by constriction, to form looped and twisted chromosomes (fig. 63). These chromosomes are at first 15 or 20 times as long as thick but later they become considerably thickened or may be nearly globular in form (figs. 65, 66).

The nucleolus retains its original size and staining character up to the time of segmenting of the spireme. When lightly stained one or more vacuoles or lighter staining areas are often visible in it (figs. 59, 63). Between the time of formation of the chromosomes and the completion of the spindle the nucleolus, as a unit, disappears though fragments with the same staining qualities are still seen within or without the nuclear area (figs. 65, 66, 67). It may be recalled here that similar dark stained granules are visible in the cytoplasm while the nuclear wall is still intact (figs. 60, 62, 64). Hence it is not certain that any of the dark bodies seen in the cytoplasm during mitosis are of nucleolar origin. During the progress of synapsis a peculiar concave sheet or disk is found lying against the inner surface of the nuclear wall, often on the side opposite the synaptic knot (figs. 59, 60, 61, 62). This disk is .5 μ thick and often 10 μ across. This body is first seen when the nucleus is just entering synapsis (fig. 60) and was not seen after the segmenting of the spireme (fig. 63). Structures which may be remains of these disks were occasionally found lying near the poles of the spindle, but the identity of these is not certain (fig. 65). This disk stains a deep black with iron haematoxylin and a dark gray with Flemming's triple stain, and in structure it may appear in section as a clear black line or, in other cases, it is rough in outline (figs. 59, 60, 62). The morphological identity of this disk has not yet been determined. Further work on the origin and fate of this disk is planned as part of a detailed study of the whole process of reduction in the embryo sac mother-cell.

A marked segregation, of the at first nearly homogeneous cytoplasm of the mother-cell, forming 2 concentric layers around the nucleus, usually occurs during the progress of synapsis. The outer layer, of 4 or 5 μ in thickness, is highly vacuolated, stains weakly and

apparently plays only a minor part in connection with the divisions of the nucleus and cell (figs. 60, 62, 63, 64). The inner layer of cytoplasm is but slightly vacuolated, is densely granular, stains darkly and becomes highly active at the time of nuclear division. This is indicated by the numerous strands and fibers formed in it (figs. 63, 65, 67, 68, 70). With the growth of the embryo sac, after the disappearance of the cell walls between the first four nuclei, this differentiation of the cytoplasm becomes gradually less marked (figs. 73, 74) and with the increased vacuolation of the eight-nucleate sac this differentiation of the cytoplasm becomes scarcely discernible (figs. 76, 77). When, however, in the sixteen-nucleate sac, the egg and synergid nuclei are cut off, and the endosperm-forming nuclei are grouped closely together, the cytoplasm about the egg and synergids and about the group of endosperm nuclei again becomes denser (figs. 84, 88). At no later time, however, does it show evidence of the extreme activity seen during the two reduction divisions. When the mitotic figure is organized in the embryo sac mother-cell, after the occurrence of the process of synapsis and chromosome-formation described above, the axis of the spindle may be either longitudinal or transverse to the ovule (figs. 65, 66). This spindle is bipolar at the earliest stage seen, acute at the poles and about $15\ \mu$ long by $10\ \mu$ across. The chromosomes are more or less bent rods, only about twice as long as thick, and are about 12 or 14 in number. In the later anaphase the chromosomes are seen to be somewhat angular but nearly isodimensional. They then measure 1 to $1.5\ \mu$ in diameter and each has attached to the side turned toward the pole a bundle of smaller fibrils (fig. 65).

The daughter nuclei from the first division are apparently organized very promptly after the chromosomes reach the poles. These nuclei are 7 or $8\ \mu$ in diameter, *i. e.*, about half as large as the parent nucleus in late synapsis. The chromatin of the daughter nucleus, at the time the new wall is first developed, has the form of a series of more than 12 rather angular granules. These deeply staining granules lie near the nuclear wall and are more or less connected by faintly colored strands (fig. 67). Before the nuclear wall breaks down, in preparation for the second division, the chromatin assumes a more uniformly thread-like arrangement and, though larger granules are still evident the meshes become more numerous and regular (fig. 68). A cell plate is often, perhaps always, formed on the spindle between these two nuclei (fig. 67).

The tetrad stage was the next stage of the development of the embryo sac of which an adequate number of examples were seen for determining the further history of these nuclei. In the young tetrads two spindles are seen usually at right angles to each other, and a well-marked cell plate is present on each (fig. 69). The nucleus at this time is 9 or 10 μ in diameter; the chromatin net is rather open and of slender threads with many small granules. The nucleolus is again evident now, for the first time since late synapsis, as a rounded, darkly staining body, about 3 μ in diameter (fig. 69). In many slightly older sacs the cell plates have been replaced by complete, though delicate, cell walls. The cytoplasm is thus cut to a tetrahedrally arranged group of cells, with (evidently), haploid nuclei (figs. 70, 71, which show adjoining sections of the same tetrad). This group has the characteristic structure, and, in the many features noted, the complete cytological history of a spore tetrad. It seems therefore impossible to escape the conclusion that this is a *tetrad of megaspores*, directly comparable with the tetrad of microspores formed in the anther of this *Peperomia* and with the linear row of four megaspores formed in many angiosperms. (See Johnson, 1907, p. 1; Coulter, 1908, pp. 363-4; Brown, 1908, p. 453). In only a few of the many cases observed were the four nuclei and the resulting cells arranged more nearly in a row (fig. 72). The question arises here whether this tetrad of megaspores is a primitive, fern-like feature, retained by this *Peperomia*. If so from what ancestor may it have been derived? On the other hand, if it is secondary in origin to what influence may this return from the serially arranged megaspores characteristic of nearly all seed plants to the tetrad arrangement found in pteridophytes be attributed? The consideration of these questions may be taken up later on, in our general discussion of results.

The walls separating the young megaspores are very delicate, so delicate in fact that one sees at first only a cleavage plane, made more evident by a slight shrinkage (fig. 70, 71). The wall itself can, however, usually be discovered in these cases, as a dark line next to one cytoplasm or the other. At a slightly later stage of development these delicate tetrad, or spore walls have disappeared and the cytoplasm once more becomes a continuous mass throughout the embryo sac (figs. 73, 74). That is the protoplasts of all four megaspores fuse to form one composite mass of continuous cytoplasm, enclosing the four megaspore nuclei (see Lloyd, 1902; Brown, 1908, p. 449; Fisher,

1914, p. 143). It is quite possible that walls are not formed in all tetrads, though it is surely the rule. In figure 69, *e. g.*, is shown a sac of large size in which the central vacuole has begun to form and in which the cell plates seem to be disappearing without giving rise to walls. From this first divergent step onward the development of this embryo sac differs greatly from that of other angiosperms, the chief peculiarity being that all the descendants of all 4 megaspore nuclei participate in forming the mature embryo sac.

The next step toward the development of the ripe embryo sac is the formation of a mitotic spindle by each of the four free nuclei. These appear very soon after the disappearance of the megaspore walls and before any other change occurs in the sac except a slight growth in length, a separation of the 4 nuclei and the formation of a central vacuole (figs. 73, 74). The spindles are located in the peripheral portion of the cytoplasm and the axis usually lies parallel to the neighboring portion of the wall of the sac (figs. 75, 76). The axis of the spindle may have any position in relation to the axis of the sac itself. The eight nuclei resulting from this division were connected, in many of the cases seen, by the persistent fibers of the four spindles. No cell plates could be found, however, nor could evidence be obtained of the separation of these 8 nuclei by even temporary cell walls (figs. 76, 77). This absence of plates, as has been pointed out by Brown (1908, p. 445), indicates that this is the equivalent of the free nuclear division found at the germination of the megaspore of most angiosperms. Smith (1911, p. 216) finds however that a cell plate is formed at the first division of the embryo sac of *Clintonia*. The eight resting nuclei at this stage are each 8 to 10 μ in diameter. Each has a nucleolus 2 or 3 μ in diameter and a wide-meshed, peripheral chromatin net not unlike that of the spore nuclei (fig. 77). During the division of the megaspore nuclei the sac increases in size and at the 8-nucleate stage has a width of 40 or 45 μ and a length of 60 μ or over. The micropylar end of the sac is narrowed to a pocket of less than half the diameter of the opposite end. In this micropylar pocket lie two nuclei derived from the nucleus of the micropylar spore of the tetrad, while just below the middle lie the three pairs of nuclei derived from the other three spores (fig. 77). The embryo sac at this stage has become more distinctly bipolar than in the four-nucleate stage, and it continues from this time on to the ripe seed to show this definite polarity.

The early phases of the preparation of these 8 nuclei for the next division have not been seen. The late telophases of this mitosis, which were seen, show 16 reorganized nuclei, connected in pairs by strands, which are evidently spindle fibers, closely like those shown in the 8-nucleate stage (figs. 76, 77). When these 16 nuclei are first formed, 4 of them lie in a pretty compact group in the micropylar pocket of the sac. The other 12 nuclei lie near or below the middle, often in 3 distinct groups of 4 each (fig. 81). It is clear from the arrangement of these nuclei that the 4 at the micropylar end have come from the micropylar spore and each of the other quartets from one of the other three spores. In a series of thin sections it is not always evident that the 16 nuclei are grouped in tetrads, but careful study of the series usually shows them to be so. At a slightly later stage of development two of the micropylar nuclei are found surrounded by denser protoplasm and cell walls, thus forming two cells that fill this end of the sac. The larger of these, the egg, has about twice the bulk of the second, which is the single synergid. In some cases these two cells may lie side by side (figs. 82, 89, 104) while in other cases the synergid may lie somewhat above the egg (figs. 84, 88, 98, 102). The egg in the mature embryo sac is often $25\ \mu$ long, and has a series of vacuoles outside the denser zone of cytoplasm immediately surrounding the nucleus. The nucleus of the mature egg may be $12\ \mu$ in diameter and has a distinct, rather fine chromatin reticulum and one nucleolus (fig. 81). The synergid is somewhat similar in organization but is usually smaller and has a less dense cytoplasm (figs. 81, 82, 84, 88). The remaining 2 of the 4 micropylar nuclei usually move downward, at about the time the cell walls appear around the egg and synergid. Later these two free nuclei are found close to the other 12 nuclei that lie in the lower half of the sac. In a few of the cases all four of the micropylar nuclei were still lying in the pocket after fertilization had occurred. Cell walls could not be seen about them and their exact origin and relation to each other could not be made out (fig. 87). It is of course conceivable that one or more of these nuclei may have come in from the pollen tube.

These 14 nuclei of the lower end of the sac at first lie in one or two groups in the peripheral sheet of cytoplasm that surrounds the single, large, central vacuole (figs. 80, 83). Later the cytoplasm becomes more abundant and the vacuoles smaller, more numerous and more peripheral in position. The nuclei are then found imbedded in a

larger, denser mass of cytoplasm that usually lies near the center of the embryo sac (figs. 82, 84). Thus far there is no indication, with one or two possible exceptions to be mentioned later, of the separation of any of these 14 nuclei from their fellows by cell walls. Not even cell plates have been found. But it must be noted that no cell plates were actually seen in the cases of the divisions forming the egg and synergid, whose walls probably arise from a cell plate, as Brown (1908) has found them to do in the case of *Peperomia sintenisii*. It is therefore possible that embryo sacs of just the proper stage would show more or less definite cell plates on the spindles of the sac in this last division. Such walls, if present, would correspond to the cell plates that form the walls of the peripheral cells of *Peperomia pelucida* (Johnson, 1900a) and of *P. sintenisii* (Brown, 1908). Such peripheral cells are rarely formed in our species, only 3 or 4 instances being noted (figs. 85, 105).

3. *Fertilization and formation of the endosperm nucleus.*

At the stage just described with two micropylar cells and the more central group of 14 nuclei, the embryo sac is mature and ready for fertilization (figs. 78, 80). The 14 nuclei may possibly not become closely grouped until after the male nucleus has entered the egg. At least, since the earlier phases of fertilization were not observed, it can only be said that all cases where the male nucleus was present in the egg the endosperm nuclei of the sac were closely grouped. The finding of structures in the style which can be identified with nothing but pollen tubes makes it practically certain that fertilization is accomplished in the ordinary way by the entrance of one male nucleus into the egg. A few cases were noted in which two nuclei were present in the egg after the penetration of the pollen tube into the micropyle. Many other eggs were seen with a nucleus containing two nucleoli (figs. 82, 83, 84, 98, 104) or a single nucleolus of double size (figs. 88, 89, 102). The nucleolus is always single except in these presumably fertilized eggs and in the primary endosperm nucleus or its derivatives. All these facts indicate that fertilization is normal in this *Peperomia*. No evidence was discovered that the second male nucleus enters into the endosperm-forming complex. This phenomenon has been sought for in each species studied but without discovering it in any of them.

As the nuclei become compacted into the group that form the endosperm nucleus the cytoplasm immediately about them grows

denser. In the cytoplasm throughout the whole lower portion of the sac there appear at this time numerous droplets of oil, varying from 1 to 6 or 7 μ in diameter. These bodies are deep black, in material fixed in Flemming's fixing fluid, and then sectioned and examined without decolorizing. They take the same color when sections of seeds preserved in formalin are treated with dilute osmic acid. This staining quality together with their behavior toward alkanna and their structure, leave little doubt that they are oil globules that probably serve as a temporary reserve food material.

Unless fertilization occurs in an egg, which may be indicated by the number or size of its nuclei and nucleoli, development throughout the whole embryo sac, and indeed in the whole seed and fruit, ceases completely. Considerable numbers of ovules in which the sacs have ceased development and have begun to degenerate, are seen on spikes where other ovules are developing normally. More rarely all, or nearly all of the ovules initiated degenerate before passing far beyond the 16-nucleate stage.

The next evident step in the further development of the embryo sac after the appearance of two nuclei in the egg is the formation of the huge endosperm nucleus. The fusion of the 14 nuclei which give rise to it is usually preceded by a crowding together of these components, until many of them are flattened against each other on one or more sides (figs. 84, 86, 87). Occasionally fusion of the nuclei in pairs may begin while the different pairs are still far apart about the vacuole (fig. 83). As the nuclei of the central group, formed in the more usual type of fusion, become compacted those in the center of the group begin to lose identity by the disappearance of their walls where in contact. There are thus formed oval or lobed nuclei, with two or more nucleoli (figs. 83, 85, 86, 87). The number of lobes, and especially the number of nucleoli, indicate clearly the number of nuclei that have fused. To the first fusion products, made up of 2 or 3 nuclei each, other single nuclei may be added or two of the fusion products may themselves melt together. Thus gradually, all of the 14 nuclei lose their individuality in the at first lobed, and often vertically flattened, primary endosperm nucleus (figs. 87, 88, 89).

Usually, at the time the nuclei come into contact, the chromatin has the form of a reticulum, though at one stage there seems to be a rather simply folded or looped chromatin thread or skein (figs. 84, 86). At the surfaces where the nuclei are in contact there is soon evident a distinct

massing of the chromatin threads. This is first seen over the area of contact, and then, as the walls between the two nuclei disappear, the chromatin becomes especially abundant in a ring about the opening through the perforated wall (figs. 87, 93, 94). Thus the chromatin reticulum does not appear to degenerate over the surface of contact, but opens out and comes to lie chiefly on the surface of the new nucleus. Part of the chromatin may, for a while after fusion, be found in the interior of the fusion nucleus. Finally the composite chromatin net comes to be pretty evenly distributed over the surface of the large endosperm nucleus. This reticulum may at first show meshes of very different sizes in different parts. These correspond in size to the different sizes of mesh seen in the different contributing nuclei before fusion. They indicate clearly that the chromatin contributed by each fusing nucleus maintains its identity for some time at least, in the primary endosperm nucleus so produced (fig. 84). Whether the smaller of these fusing nuclei play an equal part with others in the later activity of the fusion product was not determined. The fusion of nucleoli does not occur as quickly, is not carried as far as that of the nuclei, and there are always several, often 6 or 8 nucleoli in the endosperm nucleus. The number of these nucleoli present and the size of some of them indicate that the larger ones are made up of 2, and sometimes of 3 or 4 fused nucleoli (figs. 84, 86, 88). Before the fusion of the nuclei is completed, however, the number of the nucleoli begins to increase, until sometimes nearly 30 are present. These new nucleoli are apparently formed by simple budding of the large fusion nucleoli (figs. 92, 95).

4. *The endosperm and embryo.*

The egg, and likewise the whole contents of the embryo sac and seed, degenerate, as noted above, unless fertilization is accomplished. The occurrence of fertilization is indicated by the presence of two nuclei, of two nucleoli, or of a double-sized nucleolus in the egg and by the formation of the composite endosperm nucleus. The next evidence of further development in the sac, aside from the continued growth of all its parts, is the division of the endosperm nucleus. The first division of the egg itself does not usually occur until 8 or 10 endosperm cells have been formed.

The primary endosperm nucleus, when the fusion of its 14 constituent nuclei is finally completed, is a rounded, though often somewhat flattened and lobed structure. It is very large, having a diameter of 20 to 25 μ . It has a rather coarse and darkly staining, super-

ficial chromatin reticulum, and, before its first division may have as many as 25 or 30 nucleoli. The latter differ greatly in number, size and shape which differences are evidently due to the fragmentation noted above of the 14 nucleoli contributed by the fusing parent nuclei (figs. 92, 95). The resting period of this nucleus is apparently not a very long one. Relatively few examples were found where the constituent nuclei were completely fused and the product so formed was still undivided. There were many scores of examples of fusing nuclei to a few dozens of those undivided or in division.

The first spindle organized in this fusion nucleus is a very large one, with rather broad ends and very numerous chromosomes, which are crowded together at the equator to form a plate often 15 or 20 μ in diameter (figs. 98, 99). The axis of this spindle is usually approximately transverse to the ovule and the dividing wall which is at once formed on its equatorial plate is nearly longitudinal to the ovule (figs. 98, 99, 102, 105). This wall, however, may make any angle with the sagittal plane of the ovule itself. The number of chromosomes formed on this spindle and on the later ones of the endosperm is far greater than in any other mitoses of this species. They are, however, so crowded that it was impossible to count them with certainty. In all cases where the number could be made out approximately it was found to be far above a hundred (figs. 98, 99, 106). This makes it altogether probable that the 144 or 196 chromosomes of the 14 contributing nuclei retain their individuality in the resulting endosperm nucleus. The form of the chromosomes is at first rod-like, rather elongated and sometimes bent. Later they become shortened and rounded (figs. 98, 99). The two new nuclei, which are promptly organized about the poles of the spindle, are but slightly smaller than the endosperm nucleus. They are usually decidedly flattened in the plane of the new cell wall which is immediately formed between them. They are also often more or less lobed in the manner of the primary endosperm nucleus (figs. 102, 103). In fact the wall of the daughter nuclei is often so very irregular at first that in sections it looks as if torn or incomplete (figs. 102, 105). The chromatin net of these nuclei resembles that of the primary endosperm nucleus, except that it is more nearly homogeneous, in size of threads and meshes. During the first mitosis the nucleoli disappear (figs. 98, 99), but they soon reappear in the daughter nuclei, in numbers ranging up to a dozen or more each (figs. 102, 106). As in the later phases of the primary nucleus so here

the nucleoli differ greatly in size, some having ten times the diameter of others (figs. 103, 104, 105).

The spindles for the second mitosis in the endosperm are usually nearly longitudinal, and the walls formed are somewhat transverse to the ovule (fig. 104). The formation of the cell wall between the nuclei, in this as in the first and in all later divisions in the endosperm, follows immediately on nuclear division. The four endosperm cells resulting from the second division usually divide next by spindles perpendicular to those of that division, and hence by walls longitudinal to the ovule. Later a number of more or less transverse walls appear and there is thus formed an endosperm which, in the ripe seed, consists of about 40 or more cells arranged somewhat regularly in 3 or 4 tiers (fig. 108). The numbers just given are for the endosperm and embryo shown in figure 108, which is the largest found among scores of the ripest attached or fallen seeds collected by the writer. In figure 110 are shown the endosperm and embryo from a seed that was 10 or 12 days in damp moss after being collected, before being dropped into formalin and then sectioned. The embryo is farther developed than the one in figure 108, and the endosperm shows 27 cells in the single section. It seems probable that the difference in stage of development of these two seeds should be counted as due to the beginning of germination in the latter, though it is possible that development may sometimes go this far before the fruit falls from the plant. In the ripe seed these endosperm cells have irregular nuclei of 10 to 20 μ in diameter, with numerous nucleoli, and have rather densely staining protoplasts. Scattered through the cytoplasm of the endosperm, from the one-celled stage onward, are found quite numerous rounded bodies of the size of the nucleoli, which, like those of the mature embryo-sac, are colored black by Flemming's fixing fluid. This fact, together with their appearance, and their continuity from the mature sac onward, indicates that they are of the same sort of oil or fat that was demonstrated in the sac. These globules seem to diminish in size as the endosperm cells multiply. In the mature endosperm of this species, as in *P. pellucida*, stored starch, so far as the iodine test shows, is entirely wanting.

The embryo.—From the time the 14 contributing nuclei are grouped for the formation of the primary endosperm nucleus until their fusion is completed, two nucleoli are found in the single nucleus of the egg. No cases were found where the distinct male and female nuclei could

be seen in the oospore, as they were in *P. pellucida*. The two nucleoli of the oospore remain distinct and separate until the first division of the endosperm nucleus has been completed, at about which time they apparently fuse to one. The nucleus and nucleolus of the oospore both remain single from this time onward, usually until 10 or 12 endosperm cells have been formed (figs. 84, 104).

The oospore continues to grow and, at some time after the number of endosperm cells has reached 8 or 10, it begins to divide by successive walls whose sequence has not been made out. There is thus formed a small embryo which in the ripe seed shows but 8 or 10 cells in a longitudinal section (fig. 108). In the seed referred to above, which had passed two weeks in damp moss after being gathered, the form of the embryo is some what more elongated and the cells are more numerous. This probably represents the more usual form of the embryo, for this figure was drawn from sections of formalin-fixed material, which gave no evidence of shrinkage such as was seen in nearly all older embryos that had been through alcohol (fig. 110). The shaded cell at the right of the embryo in the latter figure is probably the persistent synergid. The condition of embryo and synergid in the ripe seed is thus seen to be similar to that found in the seed of *Peperomia pellucida* (See Johnson, 1900, figs. 12, 13, 14).

F. GERMINATION OF THE SEED

After searching repeatedly for germinated seeds in the field, and attempting to germinate collected and partially dried seeds in the laboratory, success was at last attained with seeds that were placed in damp moss at once on gathering, and mailed from Jamaica to Baltimore. These reached Baltimore on February 11, 1914, and were at once placed between layers of damp filter paper in a closely covered glass jar. On March 25 the radicle of the embryo was seen projecting from several of the fruits (cf. Johnson, 1902, fig. 36). In the three weeks following some of the seeds had developed a hypocotyl twice as long as the seed and a primary root three times as long (fig. 112). Examples of all stages were fixed and preserved for surface study and sectioning.

The first external indication that germination is going on is the pushing out of the wall of the fruit at the upper end. Soon the white, pearly layer of the endosperm becomes visible between the 4 or 5 parted flaps of the carpellary tissue (fig. 112). At first the endosperm

forms a complete jacket about the embryo and is commonly capped by the pushed-off stelar remnant (fig. 111). Somewhat later the tip of the radicle bursts through the micropylar end of the endosperm and continues to grow outward till the root cap, the primary root and at length the hypocotyl are differentiated and exposed (fig. 112). During all this time the endosperm remains tightly clasping about the cylindrical primary axis of the plantlet like a collar (fig. 112, and Johnson, 1902, fig. 36). Ultimately the cotyledons slip out of the clasping endosperm and spread apart to allow the expansion of the rudiments of the plumule at the stem growing point, just as in *P. pellucida* (Johnson, 1902, figs. 38, 39). The outer surface of the embryo itself shows at the very tip of the root, at a time shortly before the cotyledons have escaped, a delicate root cap about two diameters long. Behind this there are scores of root hairs of lengths reaching up to a millimeter or more. Then, at the upper limit of root hairs, there is an abrupt swelling of the axis to form the hypocotyl, which has nearly twice the diameter of the root. The surface of the usually sharply bent hypocotyl is sprinkled with hydathodes and with still more abundant oil-containing cells but no stomata are visible at this early stage (fig. 112). The cotyledons at the time of their escape have an epidermis of rather wavy margined cells with stomata, hydathodes and oil cells scattered among them.

The study of sections of the embryo during germination shows that the embryo is differentiated in the usual way from the rounded cell mass present in the ripe seed. This globular body elongates, and broadens at the chalazal end. Then two lateral prolongations of this same end give rise to the cotyledons and leave between them a depressed area from which the stem growing point is soon formed (fig. 111. See also Johnson, 1902, figs. 34, 35). The vascular system consisting of a single axial bundle from the tip of the root to the base of the cotyledons, is developed in the usual way, so far as followed. The mode of transition was not followed out because of the lack of satisfactory series of sections of this region. The behavior of the endosperm during germination is very similar to that already pointed out by the writer in *Saururus* and in other *Piperaceae* (Johnson, 1900b, figs. 7-10, and 1902, figs. 30-39). The growth of the endosperm is more active at the sides and it elongates most in the direction of the axis of the seed (fig. 111). The micropylar end of the endosperm closes together partially, at first, about the tip of the radicle. The

chalazal end, which in the ripe seed consists of 3 or 4 layers of cells below the embryo, very soon thins out to leave but 1 or at most 2 layers to separate the tips of the two cotyledons from their food supply in the perisperm (fig. 111). Whether this latter thinning out is due entirely to the crushing and absorption of the cells of this part of the endosperm by the embryo, or may be due in part also to the displacement of these cells was not determined with certainty. It seems to be due chiefly to the former process. This thin layer of tissue, between the absorbing tips of the cotyledons of the embryo and its food supply in the perisperm, has an evident advantage in allowing the more ready transfer of this material to the embryo.

The character of the contents of the chalazal endosperm cells remains similar to that of the endosperm cells of the mature seed. The protoplasm is dense with small vacuoles and the nuclei are large, characteristically irregular in shape, and may have 8 or 10 nucleoli each. It seems clear from their persistence in this position, and from the characters just mentioned that these cells must serve to pass on nutritive material from the perisperm and it is highly probable that they serve the further function of actively absorbing and digesting the contents of the perisperm cells. In other words the scantily developed endosperm, though containing little stored food itself, continues to serve as nurse for the embryo till the supply of food in the perisperm has been handed over to the young sporophyte and the rooted seedling becomes self-dependent (see Johnson, 1902, p. 334). The middle portion of the barrel-shaped portion of the endosperm remains thickest and serves as a plug to stop tightly the opening in the disrupted end of the fruit, and so probably completely prevents the entrance of water or fungi from without and likewise the escape of any dissolved food material present between the endosperm and perisperm. The cells of the upper exposed half of the endosperm mass, especially the outer ones, are much larger than those below. They also have much larger vacuoles and where exposed have thick outer walls. These characters all seem to make this part of the endosperm jacket a more adequate protection against desiccation or other injury to the delicate embryo within (fig. 111).

When the endosperm has reached a length of 600 or 700 μ it ceases to grow, and as the embryo continues its elongation the radicle is forced through the opening in the endosperm at the micropylar end, pushing aside such remains of the parietal tissue and style as may have been clinging to this end of the endosperm jacket.

G. ABNORMAL EMBRYO SACS

In addition to the exceptional occurrence of peripheral cells and of a second synergid, which were referred to in discussing the normal development of the embryo sac, because they show a resemblance, of *P. hispidula* to other species, there is a less common, probably decidedly rare, aberration still to be mentioned. This has not been described or figured in discussing the normal development for the sake of avoiding confusion. This peculiarity is the occurrence of archesporial cells, megaspore tetrads and embryo sacs in pairs or trios in the same nucellus. The occurrence of more than one archesporial cell in a nucellus has been noted in a large number of species of angiosperms since they were discovered by Strasburger (1879) in *Rosa*, and by Fischer (1880) in a half dozen other genera of the Rosaceae. Not only is this true, but in the cases of *Fagus*, *Corylus* and *Carpinus* (Benson, 1894), of *Delphinium* and *Senecio* (Mottier, 1893, 1895), and some others, two or more embryo sacs may even mature until ready for fertilization.

The sort of doubling of the megaspore and embryo sac just referred to occurs somewhat rarely in *P. hispidula*. In the youngest stages seen parietal cells were already formed. The most frequent type of doubling found is that where the twin megaspore mother cells or embryo sacs are side by side and each has essentially the structure of the ordinary single cell or sac (fig. 113). In other cases, however, two archesporial cells may lie one above the other in the axis of the ovule (fig. 114), and, still more rarely three of these cells may occur in one longitudinal row (fig. 115). The appearance of these groups of cells, especially the pairs or trios in longitudinal rows, suggests of course the possibility of there being rows of megaspores formed by the division of a mother-cell. But this possibility is practically excluded by the fact that the stage shown in figure 113 is too early to show such a division, while in the cases given in figures 114 and 117 the nucleus of one cell in each case shows clearly the typical synapsis characteristic of the maturing megaspore mother-cell. There can be no doubt that the cases mentioned really have twin megaspore mother cells. It is probable also that the cases shown in figures 115 and 116 are really groups of megaspore mother cells rather than of megaspores themselves.

The further fate of these multiple archesporial cells is also proof of their real nature. A considerable number of examples were seen of the further development of the paired archesporial cells of both types. Thus figure 118 shows two superposed embryo sacs which have evi-

dently come from an archesporial pair like that shown in figure 114, one of which has given rise to a sac with four megaspore nuclei, while the other has six nuclei in it. Sometimes the disparity in rate of

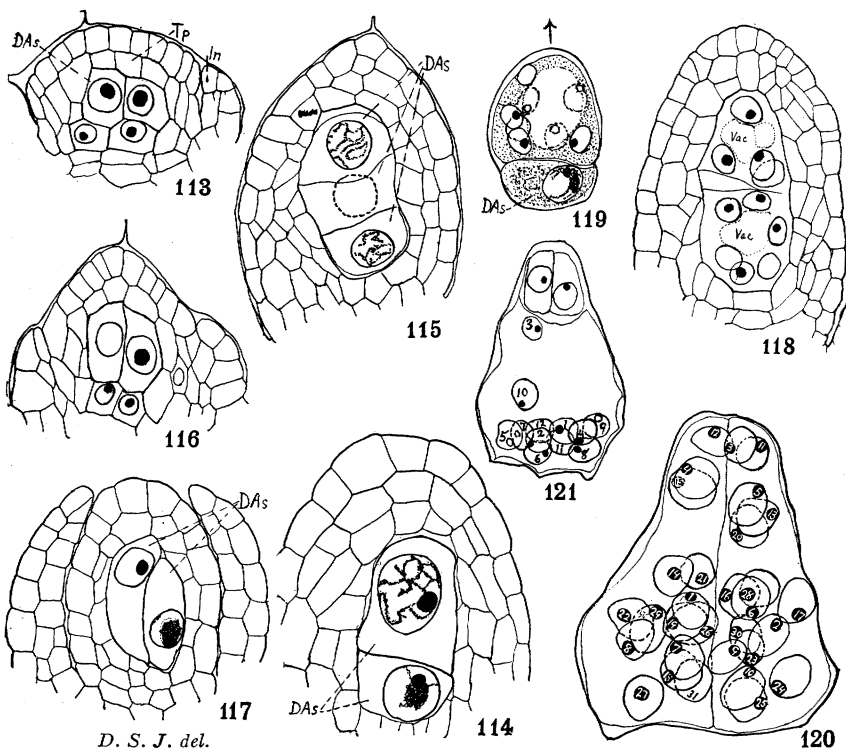


FIG. 113. Longitudinal section of a young ovule containing 2 or possibly more, embryo sac mother-cells in a transverse row. $\times 400$.

FIG. 114. Similar section showing 2 mother-cells in the axis of the ovule, the lower one in synapsis. $\times 600$.

FIG. 115. Similar section with 3 mother-cells on the same axis. $\times 400$.

FIGS. 116, 117. Similar sections of the type shown in figure 113. $\times 400$.

FIG. 118. Similar section of the type of abnormal sac shown in figure 114. The upper sac has but 4 nuclei while the lower has 6. $\times 400$.

FIG. 119. A slightly oblique longitudinal section of a later phase of the same type. One sac has 8 nuclei, the other is uninucleate. $\times 400$.

FIG. 120. Longitudinal section of nearly mature embryo sacs, one with 15, the other with 16 nuclei. These are numbered in the order of appearance of their nuclei in focus. $\times 400$.

FIG. 121. Similar section of a mature embryo sac, containing but 14 nuclei altogether. $\times 400$.

development is still greater as in the case caught by the somewhat oblique section shown in figure 119. All of the mature or nearly mature twin sacs seen were of the collateral type shown in figure 120, in which each of the embryo sacs, except for the presence of but 15 nuclei in the left one, has exactly the structure of the normal single sac at this stage. The lack of one nucleus out of the 16 in the last sac referred to exemplifies another type of abnormality not uncommon in the otherwise normal single sacs. It is possible of course that the failure to find 16 nuclei in certain evidently mature sacs may be due to the difficulty of counting the crowded endosperm nuclei. This difficulty is increased by the fact that the nucleoli, which must be largely depended on in counting, may occasionally be displaced from the surface of the section and lost. Embryo sacs are not uncommonly found in which but 13, 14 or 15 nuclei can be counted even when the egg and synergid are differentiated. Figure 121 shows such a sac in which but 14 nuclei could be discovered, including those of the egg and synergid.

The ultimate fate of the paired embryo sacs mentioned has not yet been determined. From the occurrence of a number of cases like that shown in figure 120 it seems probable that two eggs may be fertilized in one ovule and thus two embryos be formed. No case of this sort has been seen in *P. hispidula*, but seeds of *P. pellucida* have been seen from which two embryos protruded on germination. It may be discovered that these are developed from separate twin embryo sacs in the same ovule, such as have been described for *P. hispidula* but have not yet been reported for *P. pellucida*.

The occurrence of these abnormal embryo sacs gives us no indication of the phylogenetic origin of the peculiar composite sac normally found in this and other species of *Peperomia*. Neither of the two methods of doubling here found can be conceived of as in any way explaining the origin of the simple 16-nucleate sac from the 8-nucleate one characteristic of most angiosperms. This is evident from the fact that each of the twin mother cells, by itself, gives rise to a complete 16-nucleate embryo, and from the fact that in both the normal case and in the case of the twins it is one complete megaspore tetrad that gives rise to each complete individual sac.

The widespread distribution among the angiosperms of the genera in which these multiple archesporial cells and embryo sacs occur is practically conclusive evidence that they can have no value as indicators of phylogeny aside from supporting the well-established view

that all angiosperms are derived from archegoniates with a many-celled archesporium in the megasporangium.

Finally it might be noted that though the causes producing these abnormal double sacs were not discovered it was found that the causes seem evidently confined to relatively few plants. Each of the abnormal plants may bear several of the double embryo sacs in the same spike, along with normal ones. The mature embryo sacs with two or three nuclei less than normal probably owe their peculiarity to some minor disturbance of nutrition which inhibits nuclear division before all of the megaspore nuclei of each sac have completed the second division.

THEORETICAL CONCLUSIONS

The peculiarities in the structure and development of the vegetative and reproductive organs of *P. hispidula* recorded above lead to certain conclusions concerning the meaning and phylogenetic origin of these peculiarities. The most important secondary question which these facts may help to solve is that of the comparative primitiveness of this plant in relation to other members of its genus and family and to other angiosperms.

We will consider the bearing of the facts here recorded under the following heads: (1) Vegetative structure. (2) Structure and development of the stamen flower and fruit. (3) Development of the archesporium and megaspores (cell plates etc.). (4) Development of the embryo sac including division of megaspore nuclei, formation of the egg apparatus, endosperm nucleus and peripheral cells. (5) Development of embryo and endosperm. (6) Germination and food-storage in the perisperm. (7) Relative primitiveness of the 8-nucleate and 16-nucleate embryo sacs.

1. *Vegetative structure*.—As was stated above, the structure of the vegetative organs of *P. hispidula* approaches in many features the simplest types known for these organs among dicotyledons. This is shown not only in the habit or organization of the plant as a whole but by the intimate structure of each of the individual organs. Thus the root is small, sparsely branched and has a single very simple vascular bundle. The stem likewise is relatively short and little-branched, is delicately herbaceous and has a simple internal structure, although the single, closed, vascular bundle is slightly more complex than that of the root. The leaf though furnished with trichomes, hydathodes and oil-cells on the surface has, aside from its three water glands, the simp-

lest sort of internal structure with slightly specialized palisade cells and but one, or in places two other layers of mesophyll cells. All in all the internal structure of this *Peperomia* is the simplest thus far described within the genus. This means also that it is the simplest within the family.

The question arises as to whether this simplicity is primitive or not. The more generally accepted views of the character of primitive angiosperms, which were probably dicotyledonous, conceive them to be tree-like or shrub-like woody plants (Wettstein, 1911, p. 196; Arber and Parkin, 1907; Maneval, 1914; Engler and Gilg, 1912; p. 156). It is clear that all these writers must regard this *Peperomia* as far removed in vegetative structure from the most primitive type for the dicotyledons. The tree-like *Pipers* though not the most primitive dicots in stem structure seem clearly the more primitive members of the Piperaceae. *P. hispidula* on the other hand must be regarded as among the most highly specialized members of this family, in most points of vegetative structure.

2. *Development and structure of flower and fruit.*—In treating these topics it will not be necessary to discuss in detail the much disputed question of the relative primitiveness of unisexual and hermaphrodite flowers among angiosperms nor to decide whether naked flowers are sometimes primitive, as well as sometimes due to reduction. One or two points showing the relative simplicity of this *Peperomia* in its own family seem reasonably clear. In the first place all the flowers are potentially hermaphrodite and there is no variation in the number of microsporangia formed in each stamen, such as occurs in some other Piperaceae (Johnson, 1910). This number, however, is but two, a very unusual number among angiosperms. The same number of microsporangia per stamen is found throughout the genus *Peperomia*, while in other Piperaceae, and in nearly all other angiosperms, four sporangia are found in each stamen. Evidently *P. hispidula*, along with the others of its genus, is decidedly aberrant or specialized in this respect. The development of the stamen, of the microspore, and of the male prothallus is like that of the vast majority of angiosperms.

The history of development indicates very definitely that the carpel of *P. hispidula* is single, as there is not the slightest trace of its having a compound origin. Yet the carpels of its nearest allies the *Pipers* show rather definite evidence, in the presence of three lobes in the stigma and of six vascular bundles in the wall of the ovary, that

the latter is composed of three carpels (Johnson, 1902, 1910). *Saururus* also (Johnson, 1900b), which is still regarded by Engler as the most primitive of the Piperales, shows a similar compound ovary. In other words, the carpel also seems simplified through specialization by reduction from the more primitive type characteristic of the Piperaceae in general. The mature carpel or fruit is thin and of few layers of tissue as compared with the *Pipers* studied. Not the slightest evidence has been found that any floral envelopes were ever present in this plant. The above-mentioned evidence points to this and other *Peperomias* as being forms with flowers more specialized than those of the related *Pipers*.

In the single ovule of *Peperomia*, we have, as has been pointed out by Fisher (1914, p. 148), another character which cannot be regarded as primitive by those accepting any of the current views of the relationship of the Piperaceae (see Engler and Prantl, p. 189; Hallier, 1905; Lotsy, 1911, pp. 487 ff.; Engler and Gilg, 1912, pp. 157, 158). It must of course be kept in mind that while the number of ovules per carpel is rather constant in some families there are others like the Rosaceae where the number may vary from one to several.

3. *The development of archesporium, megaspores and cell plate.*—The hypodermal primary archesporial cell is usually single and by its division gives rise to a definitive archesporial cell that becomes the mother-cell of the embryo sac, and above this a tapetal cell that forms several layers of tapetal tissue in the mature seed. The definitive archesporial cell and its nucleus enlarge to double their original size. The nucleus after showing a typical synapsis undergoes 2 mitoses by spindles perpendicular to each other giving rise thus to four, probably haploid, nuclei arranged at the points of a tetrahedron within the nearly spherical embryo sac. Delicate cell walls then arise from cell plates of the usual type, forming thus 4 tetrahedral megaspores, comparable in appearance and origin with the microspore tetrad of this species, or, except for the delicate character of the walls, with the megaspore tetrad of *Selaginella*. The whole development of these 4 cells, including the chromosome history of the nuclei as far as followed, shows them to be the exact equivalents morphologically of the megaspores, which in most angiosperms are arranged in an axial row (see Coulter, 1908, p. 364). The unusual arrangement of megaspores in a tetrad in this *Peperomia* is probably to be associated with the somewhat rounded form of the spore mother cell, and this depends,

perhaps, on the form of the ovule (see Johnson, 1900, p. 2; Brown, 1908, p. 453; Stephens, 1909*b*, p. 383; Brown and Sharp, 1911, p. 446; Fisher, 1914, pp. 421-430). That the form of the ovule and embryo sac and the arrangement of the contents of the latter may be related to each other and may vary considerably in the same plant is shown by this *Peperomia* itself. Thus when the 4-nucleate embryo sac is about isodimensional the nuclei are arranged at the corners of the tetrahedron, while in other cases where the embryo sac is twice as long as broad the nuclei are in 2 subterminal pairs (fig. 72.) Of the external and internal causes determining the form of the ovule and the associated form and arrangement of the embryo sac we know almost nothing (Brown and Sharp, 1911, p. 446).

As for the phylogenetic origin of this tetrad arrangement of the megaspores of *P. hispidula* it seems clear that it is a peculiarity developed within the genus, for the allied and probably more primitive genera *Piper*, *Heckeria*, *Saururus*, etc., have the megaspores or megaspore nuclei arranged in a row (see Johnson, 1900*b*, 1910; Fisher, 1914, p. 156). No adequate evidence has been offered to show that this megaspore tetrad has been derived directly from a more primitive ancestor of which this arrangement was typical, as it is of the megaspores of the higher pteridophytes. The same can be said of those other atypical angiosperms, having tetrahedrally arranged megaspore nuclei not separated by walls, such as *Gunnera* (Schnegg, 1902; Ernst, 1908; Samuels, 1912), the Penaeaceae (Stephens, 1909*a*) and certain *Euphorbias* (Modilewski, 1909). In none of these has the tetrahedral arrangement of the megaspore nuclei been shown to be primitive, and in each case this arrangement has been found within a rather rounded embryo sac.

4. *Embryo Sac*.—Under this head will be considered the disappearance of the megaspore walls, the further division of the megaspore nuclei, the formation of the egg apparatus, of the endosperm nucleus, and of the occasional peripheral cells.

The separating walls of the megaspores are delicate and do not persist long after their formation from the cell plates of the second division. As they disappear the cytoplasm again becomes continuous, and a large central vacuole replaces the series of numerous small peripheral vacuoles, characteristic of the earlier phases of development. This single continuous mass of protoplasm with its 4 nuclei must undoubtedly be regarded as the product of fusion of 4 megaspores, and

the single individual embryo sac thus formed as a composite female gametophyte. This sort of composite young gametophyte has been observed in *Avena fatua* (Cannon, 1900) and in *Crucianella* (Lloyd, 1902), with the difference, however, that in these forms 3 of the megaspore nuclei degenerate, sometimes after one division each, while the fourth gives rise to all of the 8 nuclei of the mature embryo sac. Lloyd pointed out the significance of this association, of the first haploid nuclei from a megaspore mother cell, in one cell and spoke of this cell as "an individual by coalescence?" Coulter (1908) applied this same criterion, the chromosome number, to the interpretation of all embryo sacs in which the first nuclei produced in the ovule by the reduction division are included, either primarily or secondarily in a single protoplast. According to this view the embryo sacs not only of *Peperomia*, *Gunnera* and the Penaeaceae, but also those of *Lilium*, *Piper* and many others where the "megaspore mother cell develops directly into the embryo sac," are to be regarded as composite sacs. The demonstration of a reduction process and of evanescent megaspore walls in several species of *Peperomia* by Brown (1908) and Fisher (1914) and the especially clear case of tetrad formation in *P. hispidula* show that this genus furnishes several of the clearest instances of the formation of a composite mature embryo sac. The possibility of other interpretations of the observed phenomena which imply that chromosome reduction may occur at points in the life history, other than the divisions giving rise to the megaspore nuclei, have been suggested by Atkinson (1901), Brown (1908) and Ernst (1908, p. 27). The study of *P. hispidula*, however, together with that of other species by Brown and Fisher make it very clear that, in this genus, a genuine megaspore-formation is associated with the reduction division, and make it altogether probable that the same is true in *Piper*, *Lilium*, and in many other similar cases.

The 2 further divisions of each of the 4 megaspore nuclei are not followed by cell walls, nor even by cell plates. These facts suggest the homology of these divisions to the first steps of megaspore germination in ordinary angiosperms (see Brown, 1908, p. 453). Though it must be recalled that cell plates are formed in the first division of the megaspore nucleus of *Lilium*, *Clintonia* and perhaps others (R. W. Smith, 1911, p. 216). The 16 nuclei resulting from the 2 divisions of the megaspores are usually grouped in distinct quartets, one of them at the micropylar end of the embryo sac. Two of the nuclei

of this micropylar group soon become surrounded by denser masses of cytoplasm and by cell walls, to form the egg and the single synergid. The other 2 nuclei of this group and the 12 of the other three groups later form a compact group near the middle of the short flask shaped embryo sac. This is the mature sac ready for fertilization. There are no 8-nucleate *mature* embryo sacs in this species as Coulter (1908, p. 363) has misunderstood me to state in my preliminary account of this species (Johnson, 1907). After fertilization, the occurrence of which is indicated by the presence of 2 nucleoli in the egg, the 14 nuclei of the central group fuse completely to form a huge, lobed, endosperm nucleus. Thus while the egg and synergid both come from one of the 4 megaspore nuclei the descendants of all 4 megaspores enter into the formation of the endosperm nucleus, making the latter the only really composite element in the embryo-sac.

From what has just been said it is evident that the striking peculiarities of the development of the embryo sac here are the occurrence of but 2 divisions of the megaspore nucleus instead of 3, the participation of all megaspores in the formation of a single sac and the organization of the endosperm nucleus by the fusion of so large a number of nuclei derived from megaspores. It seems perfectly clear, since the first 4 nuclei formed in the embryo sac are certainly megaspore nuclei, that the mode of megaspore germination here shown is not a primitive one but a specialized type reduced from one with 3 divisions such as occurs in other angiosperms. In the second place the single multinucleate prothallus or embryo sac formed by the cooperation of 4 megaspores has no analogue among lower seed plants or the pteridophytes, and therefore cannot possibly be regarded as primitive (see Campbell, 1899, p. 455, 1901, pp. 113-117, 1902, p. 781), or as recalling the many-celled prothallus of the gymnosperms and pteridophytes, which is everywhere developed from a single megaspore. Finally the formation of the endosperm nucleus by the fusion of 14 nuclei from 4 megaspores has no homologue outside of a few other peculiar angiosperms (*Gunnera*, Penaeaceae) and is pretty certainly a later specialization rather than a primitive type from which the typical endosperm nucleus, formed by two nuclei from a single megaspore, may have been derived. There is nothing about the development of this endosperm to suggest the possibility of its being a modified second embryo as suggested by Miss Sargent and others. It is to be recalled that Porsch has pointed out that no resemblance

could be expected now, even if this were its nature. There is no evidence of the fusion of a second male nucleus in the endosperm group.

The occasional formation in *P. hispidula* of peripheral cells by the enclosure of one or two of the 16 nuclei of a sac by cell walls is probably to be regarded as a partial reversion to the fate characteristic of part of these nuclei in all of the 10 other *Peperomias* studied (Johnson, 1900a; Brown, 1908; Fisher, 1914). No instances of these peripherals were seen in my earlier work on *P. hispidula* (Johnson, 1907). The agreement of the other 10 species with each other is strong evidence that the embryo-sac with half a dozen peripheral cells is the more primitive type characteristic of the genus, from which the *P. hispidula* type, with few or none, has been derived.

There seems no particular reason for comparing the 1 or 2 peripheral cells of *P. hispidula*, when formed, or the 6 peripherals of other *Peperomias* with the antipodals of other angiosperms, except in so far as they are left over after the essential structures of the embryo sac have been organized. They certainly give no evidence of being sterile egg apparatuses, such as Porsch (1907) would expect to find in a primitive angiosperm.

Concerning the physiological cause and significance of the peculiar type of organization of the embryo-sac and endosperm nucleus found in *P. hispidula* we know nothing. Brown (1908), has suggested that the globular form of the mother cell gives all four of the megaspore nuclei formed in it an equal advantage in space and food supply. But it is to be remembered that other rounded mother cells may give rise to but one functional megaspore while in other angiosperms the composite embryo sacs are decidedly elongated. Among the latter are to be counted *Gunnera*, *Lilium*, and probably *Piper*, and many others with elongated sacs can be added to this list when the point at which chromosome reduction occurs has been determined.

Of the significance of the multiple fusion in the formation of the huge endosperm nucleus nothing definite can be said except to make the oft-repeated statement of fact that where two or more nuclei are left together in one protoplast they usually fuse to one nucleus. In the present instance the effect is to practically overcome the disparity in size of nucleus and protoplast (cf. figs. 58 and 88). The only evident physiological advantage of a fusion of the 14 nuclei into a single nucleus is that of forming a large nuclear unit adequate for the control of the

large basal end of the embryo sac. Such a nucleus is perhaps especially needed for the formation of the relatively broad cell walls that are at first formed in the endosperm. Possibly also the activity of the endosperm cells in nourishing the embryo, during seed-development and at germination, is more efficient because of the presence of these large nuclei in them. But these advantageous *results* of the process do not, of course, explain its *causes* which will pretty certainly be determined only after experimental study.

5. *The development of the embryo and endosperm.*—The fertilized egg, with its two nucleoli, or its single enlarged nucleolus, remains undivided and unchanged, except for an increase in size, during the aggregation and fusion of the endosperm-forming nuclei and even during the first division of the fusion nucleus. Soon after this the oospore, or embryo, divides by a longitudinal wall and then by walls in other planes, whose sequence is undetermined, to finally form in the ripe seed, a suspensorless, slightly elongated embryo of about 50 cells.

The large, lobed, central endosperm nucleus has 20 or more nucleoli. It divides mitotically by a huge spindle whose axis is transverse to that of the sac and which has on it 144 or more chromosomes. The second and third series of walls in the endosperm are approximately longitudinal and these are followed by irregularly placed walls that form an ellipsoid mass of 100 or more cells in the ripe seed. The endosperm is cellular from the start, each mitosis from the first is accompanied by the formation of a cell plate and wall. The cells of the mature endosperm have large, lobed nuclei and rather dense cytoplasm, containing many oil globules but no starch.

This mode of development of the endosperm, in which a cell wall immediately follows each division of the nucleus, cannot be regarded as the most primitive type occurring among seed plants. I have already pointed out (Johnson, 1905, p. 31), that this mode of endosperm-formation is one not found at all among gymnosperms and occurs elsewhere among angiosperms only in forms that are generally recognized as rather highly specialized forms. This same point has been more fully elaborated by Samuelsson (1913, pp. 135-145). Moreover this *succedaneous* type of formation of endosperm cells is not encountered among pteridophytes, save in two highly specialized families, the Marsiliaceae and Salviniaceae, neither of which can be thought of as a very probable ancestor of angiosperms.

6. *Germination and the storage of food in the perisperm.*—At germi-

nation of the seed the endosperm, with the enclosed embryo, bursts out of the seed-coat and fruit and finally is itself ruptured by the radicle of the growing embryo. The tips of the cotyledons remain for a long time embedded in the cap of endosperm, which persists between them and the perisperm. From this position of the endosperm it must evidently serve as a conveyor of the stored starch from the perisperm to the cotyledons. This location of the stored starch in the sporangial tissue is by no means a very primitive feature among seed plants. It is found in no pteridophyte and in no gymnosperm, except the bare remnant of nucellus left in the seed of *Torreya*. Among angiosperms, both dicotyledons and monocotyledons, perisperm occurs only in forms that on other grounds have been considered decidedly specialized (Johnson, 1902, p. 337).

The persistence of the endosperm as a nurse for the embryo during its development and germination is characteristic of all the Piperales thus far studied and also of certain perisperm-containing monocotyledons (Johnson, 1900b, 1902). In the case of the orchids and the Podostemaceae, as described by Magnus (1913), endosperm is wanting and thus, as Magnus points out, the nutritive function of the female gametophyte is completely lost. In these two families this gametophyte serves simply to mature a functional egg apparatus. Magnus suggests that this disappearance of the endosperm allows a more rapid transfer of material to the embryo directly from the parent sporophyte, which is aided by a haustorium of the suspensor. It is not evident to the writer why this should allow more rapid nutrition of the embryo, nor is it proven that the embryos of these forms actually are developed more rapidly, in proportion to their size, than those of forms with endosperm. In the series of seed plants showing different degrees of development and functioning of the diploid generation, beginning with the gymnosperms and *Ricinus* and ending with the orchids and Podostemaceae, it is evident that *Saururus*, *Peperomia* and *Canna* show the next to the last step in the reduction of the endosperm, both in relative bulk and in variety of functions. Magnus suggests that such forms without endosperm, the Podostemaceae for example, may become the progenitors of entirely new races of seed plants. The haploid generation of these hypothetical plants might, we can imagine, come to consist merely of one of the four (or possibly three) haploid nuclei resulting from the reduction division of the nucleus of the megaspore mother-cell. It is also quite possible to conceive that one of these

nuclei, without being cut off from the others by a cell wall, might be fertilized and so give rise to an embryo which should be nourished directly by the surrounding tissue of the parent diploid generation. If this happened we should have realized in the seed plants the extreme reduction of the diploid generation which is found in animals, and perhaps in *Fucus*, but which is only approximated by the Orchidaceae and Podostemaceae.

7. *The relative primitiveness of the 8-nucleate and 16-nucleate embryo-sacs.*—The view that the embryo-sacs with 16 nuclei are primitive, in that they retain more of the numerous nuclear divisions intervening between megaspore and ripe egg, which are characteristic of the gymnosperms and heterosporous pteridophytes, is one first advanced by Campbell (1900–1901). His conclusion was based on the assumption that all of the 16 nuclei present in the mature embryo sac result from the germination of a single megaspore. The recent work on several species of *Peperomia* (Johnson, 1907, Brown, 1908, Fisher, 1914), on the Penaeaceae (Stephens, 1909a), on *Gunnera* (Ernst, 1908, Samuels, 1912) and the present study show, as Coulter (1908, pp. 363–364) has suggested, that Campbell's assumption is clearly incorrect. The individual megaspore in each of these genera gives rise not to more prothallial nuclei than in other angiosperms but to just half as many, four instead of eight. It is clear that this can hardly be regarded as a very primitive mode of megaspore-germination. It is rather a more reduced type than the one usually found in angiosperms. From this point of view then Campbell's contention that the 16-nucleate embryo sac is primitive is clearly untenable. From this point of view also it is evident that, since the embryo sac of *Peperomia hispidula* is shown to be the product of division of 4 individual megaspore nuclei, in a common cell cavity, the number of nuclear divisions between the definitive archesporial cell and the mature embryo sac is not a matter of prime significance. Since then the reduction division and megaspore formation in *P. hispidula* goes on entirely in the normal way except for the arrangement of the spores in a tetrad, we cannot agree with Ernst (1908, p. 29), that the process of tetrad division here is reduced and that the germination of the megaspore involves one more division than usual and that this sac is thereby "*distinguished as an older, or at least as an independent, form of angiospermous embryo sac.*"

It would still, of course, be possible to regard the peculiar type of megaspore-germination found in *Lilium*, *Peperomia*, *Gunnera*, etc., as

an independently developed one, if the forms possessing it were at all related genetically. It is a matter of fact, however, as has been pointed out before (Johnson, 1902, p. 336; Maneval, 1914, pp. 9, 10), that the forms possessing these composite sacs belong to widely separated families. Moreover this compound type of embryo sac may be found in but one or two genera of the family in which it occurs. Both these facts make it just as impossible to believe that the compound sac of *Lilium*, for example, is a long-established or fundamentally peculiar type as to believe that the very different peculiarities of the sac of *Tulipa sylvestris*, of *Trillium grandiflorum*, of *Clintonia borealis*, or of *Smilacina* are primitive and fundamentally peculiar in their significance. The same reasoning would also deter us from regarding the abnormalities of the sacs of *Cypripedium* (Pace, 1907), or *Epipactis* (Brown and Sharp, 1911), as other than recently acquired variations of the usual type of development of the angiospermous embryo sac.

Because of the scattered distribution of these abnormal types of embryo sac it seems quite clear that they have been independently developed. Moreover, if the 4-spored, 8-nucleate type of *Lilium* can be developed phylogenetically from the 1-spored, 8-nucleate type characteristic of its family as a whole, and therefore presumable primitive, then the 4-spored, 16-nucleate type of *Peperomia* may well have arisen from the 4-spored, 8-nucleate type of the other *Piperaceae*. That is, the 16-nucleate sac has in all probability, contrary to the opinion of Ernst, arisen from the 8-nucleate one (see Johnson, 1900a, 1902). For the same reason the single synergid of *Peperomia* must be regarded as a later, incidental modification of the usual arrangement, of no more significance than the fact that *Ornithogalum* has but one synergid while the rest of the Liliaceae have two.

SUMMARY

1. In vegetative structure *Peperomia hispidula* is the simplest described species of the genus and family. The delicate herbaceous stem, the single closed bundle in stem and root, and the delicate, gland-covered leaf, are probably not primitive features but are due to recent modification of the more complex type of structure, which is characteristic of the other members of the genus.

2. This species has hermaphrodite, naked flowers and there is no indication in its development that it ever possessed floral envelopes. There are, as in other *Peperomias*, only 2 microsporangia per stamen

instead of the more primitive number, 4, that is found in most angiosperms. The ovary is of a single carpel, with no indication of the tricarpellate condition of which there seem to be clear traces in the development of its ally *Piper*.

3. The nucleus of the definitive archesporial cell has a thin, darkly staining concave disk lying just within its wall. This cell divides, with a characteristic synapsis and reduction in its nucleus, to 4 tetrahedral megaspores. The delicate walls of these megaspores soon disappear, leaving their 4 nuclei in a single, continuous protoplast. This globular tetrad of spores is probably a recent innovation in the development of this genus. It is perhaps a modification of the linear series of spores, characteristic of *Piper* and of most other angiosperms, that is related in some way to the globular form of the archesporial cell and sac of *Peperomia*. There is surely no adequate evidence that this tetrad arrangement has come down directly from a primitive ancestral form.

4. The four megaspore nuclei divide in the single protoplast to form one compound, 16-nucleate gametophyte, or embryo sac, consisting of an egg and a synergid, which are both from one megaspore, and of a huge endosperm nucleus formed by the fusion of the remaining 14 nuclei. This compound embryo sac has no ancestral fore-runner among gymnosperms or pteridophytes and is therefore regarded as a recently specialized type. It probably has arisen independently in that genus, for physiological reasons that have not yet been made clear. Quite rarely peripheral cells are formed, but only one or two, instead of the half dozen, characteristic of the other, probably more primitive, *Peperomias* that have been studied.

5. A cell wall immediately succeeds each division of the large endosperm nucleus and an endosperm is formed of 100 or more cells, containing some oil globules but no starch in the mature seed. This type of endosperm formation, which is found in no simpler plants nearer than the Marsiliaceae, cannot be regarded as primitive. Starch for the nutrition of the embryo is stored in the perisperm. This is probably not a primitive feature in angiosperms, since it does not occur in either gymnosperms or pteridophytes and is not known in any undoubtedly primitive angiosperm.

6. At germination the small, globular embryo is enclosed and nourished by the swelling endosperm until the primary organs of the former are organized. The tips of the cotyledons remain enclosed in

the endosperm until the starch of the perisperm is exhausted. This restriction of the functions of the endosperm to that of nurse for the embryo, is the next to the last step in the disappearance of the endosperm, which has become practically complete in the Orchidaceae, the Podostemaceae and the Helobiales.

7. The 16-nucleate embryo sac of *Peperomia* like those of other atypical angiosperms cannot be regarded as primitive. In the first place it is highly peculiar in being the product of four megasporos, germinating in a single protoplast, a phenomenon unknown among simpler forms. In the second place the angiosperms with 16-nucleate embryo sacs belong to isolated genera, in many unrelated families whose other genera have 8-nucleate sacs, from which it is believed the 16-nucleate sacs have become specialized independently in each family. Thirdly: the whole vegetative and reproductive structure and development of this species favor the view that it is a specialized form among its allies.

THE HARPSWELL LABORATORY,
August, 1914.

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EXPLANATION OF PLATES XLI-XLIII

FIG. 57. Sagittal section of ovary, ovule, and part of subtending bract. Showing initiation of stigma, stylar canal, parietal cell and definitive archesporial cell. $\times 90$.

FIG. 58. Half-grown definitive archesporial cell (embryo sac mother-cell), showing character of chromatin net and cytoplasm. $\times 1,150$.

FIG. 59. Similar section of slightly older embryo sac, showing incipient synapsis and the initiation of the nuclear disk. $\times 1,150$.

FIG. 60. Similar section showing nearly complete synapsis. Nuclear disk distinct and free from nuclear wall. $\times 1,150$.

FIG. 61. Similar section showing complete synapsis. Nuclear disk distinct. $\times 1,150$.

FIG. 62. Similar section, showing double and twisted chromatin threads. Nuclear disk rough. (Nucleolus in an adjoining section.) $\times 1,150$.

FIG. 63. Similar section of slightly later stage, showing formation of chromosomes. Nuclear disk not evident. $\times 1,150$.

FIG. 64. Similar section of older embryo sac mother-cell, showing rounded chromatin masses in nucleus and large, darkly-staining granules in the cytoplasm, though nuclear wall is still intact. Nucleolus and nuclear disk not distinguishable. $\times 1,250$.

FIG. 65. Similar section showing part of spindle of first division of megaspore mother-cell. The dark rods near the poles of the spindle are, perhaps, the remains of the nuclear disk. $\times 1,250$.

FIG. 66. Part of a similar section, showing all chromosomes at a later phase of the same spindle. $\times 1,250$.

FIG. 67. Similar section showing the chromatin in the partially organized nuclei resulting from the first mitosis in the embryo sac. Cell-plate evident. $\times 1,250$.

FIG. 68. Approximately longitudinal section of ovule containing binucleate embryo sac, showing some remains of the spindle of preceding mitosis. The cell plate not distinguishable, probably because of the obliqueness of the section. $\times 350$.

FIG. 69. Longitudinal section of embryo sac showing first 4 nuclei formed, with cell-plates. $\times 1,250$.

FIG. 70. Similar section of an embryo sac, showing 3 megaspore nuclei surrounded by cytoplasm and separated by cell walls, to form megaspores in a tetrad. $\times 1,250$.

FIG. 71. The section adjoining that shown in last figure, showing the fourth nucleus and megaspore. $\times 1,250$.

FIG. 72. Similar section of a more elongated embryo sac, with part of one of cell walls. $\times 600$.

FIG. 73. Similar section of an embryo sac with 4 megaspore nuclei still connected by fibres. The central vacuole has increased in size. $\times 1,250$.

FIG. 74. Similar longitudinal section of ovule with 4 unseparated megaspores. $\times 350$.

FIG. 75. Similar section showing first mitosis of the 4 megaspore nuclei. $\times 665$.

FIG. 76. Longitudinal section of an 8-nucleate embryo sac. $\times 665$.

FIG. 77. Longitudinal section of 8-nucleate embryo sac with the nuclei in two groups. The nuclei shown in dotted outline are from the adjoining section of the same sac. $\times 530$.

FIG. 78. Sagittal section of young fruit and bract showing vascular connection with axis. Embryo sac slightly diagrammatic. $\times 100$.

FIG. 79. Transverse section of style, near middle of its length. Showing small-celled conducting tissue and large tracheae. $\times 125$.

FIG. 80. Longitudinal section of 16-nucleate embryo sac, showing differentiation of cytoplasm about egg and synergid. The dotted nuclei are from an adjoining section. $\times 530$.

FIG. 81. Similar section showing egg and synergid with walls. The nuclei of this sac are in 4 groups, showing their derivation from 4 megaspores. $\times 530$.

FIG. 82. Similar section of older sac, with 14 prothallial nuclei grouped near center, ready for fusion. Dotted nuclei are from an adjoining section. Cytoplasm between vacuoles diagrammatic. $\times 530$.

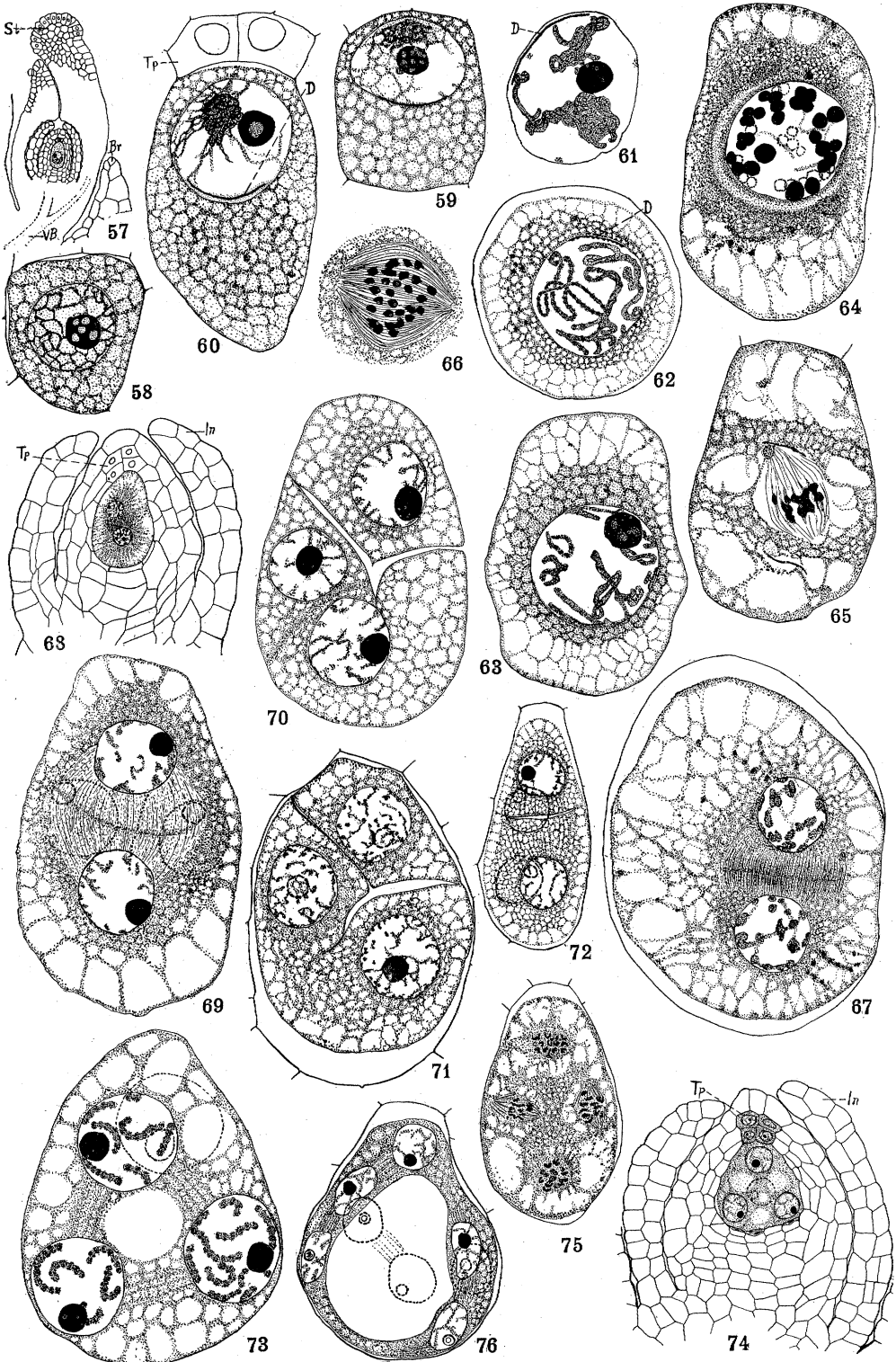
FIG. 83. Similar section of a sac in which fusion of the 14 constituents of the endosperm nucleus has begun, though they are still scattered around the central vacuole. The nucleoli of this and following figures are numbered in the order of their appearance in focus from one side of the sac to the other. Nucleoli 1 and 2 are in one fusion nucleus and 3 and 4 are in another. $\times 500$.

FIG. 84. Similar section of an embryo sac, showing 13 grouped nuclei of which the large one, numbered 11, with a large nucleolus, has been formed by the fusion of 2 of the 14 free prothallial nuclei. The cytoplasm is becoming denser about the grouped nuclei. The 2 nucleoli in the egg indicate that fertilization has occurred. The small nuclei, 5 and 6, were perhaps destined to degenerate. $\times 900$.

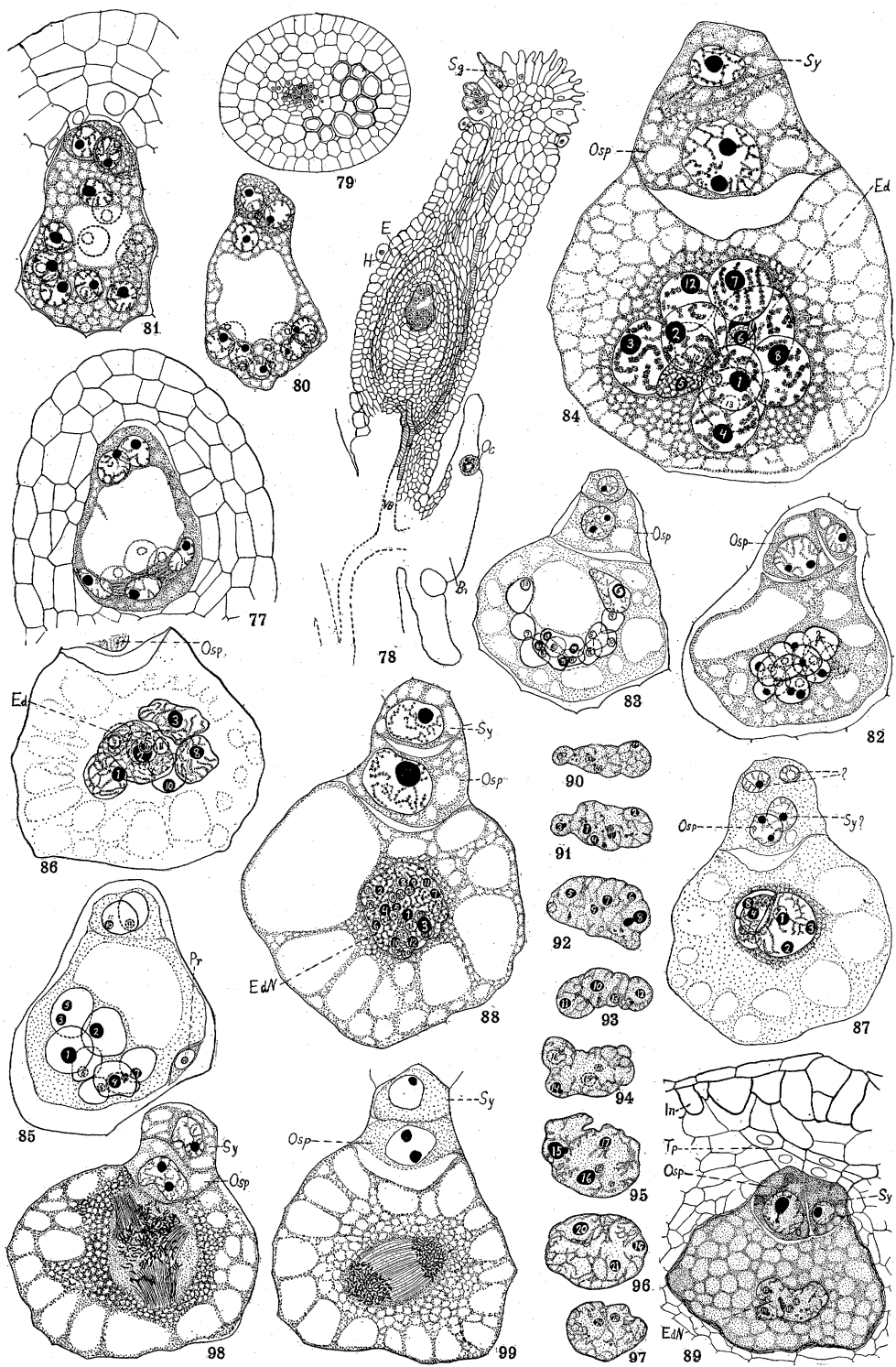
FIG. 85. Similar section showing 4 fusion nuclei in an endosperm group (those containing nucleoli 1-5). The nucleus at the right is enclosed in a peripheral cell against the wall of the sac. (Cytoplasm diagrammatic.) $\times 530$.

FIG. 86. Basal half of similar section, showing more advanced stage of fusion. $\times 530$.

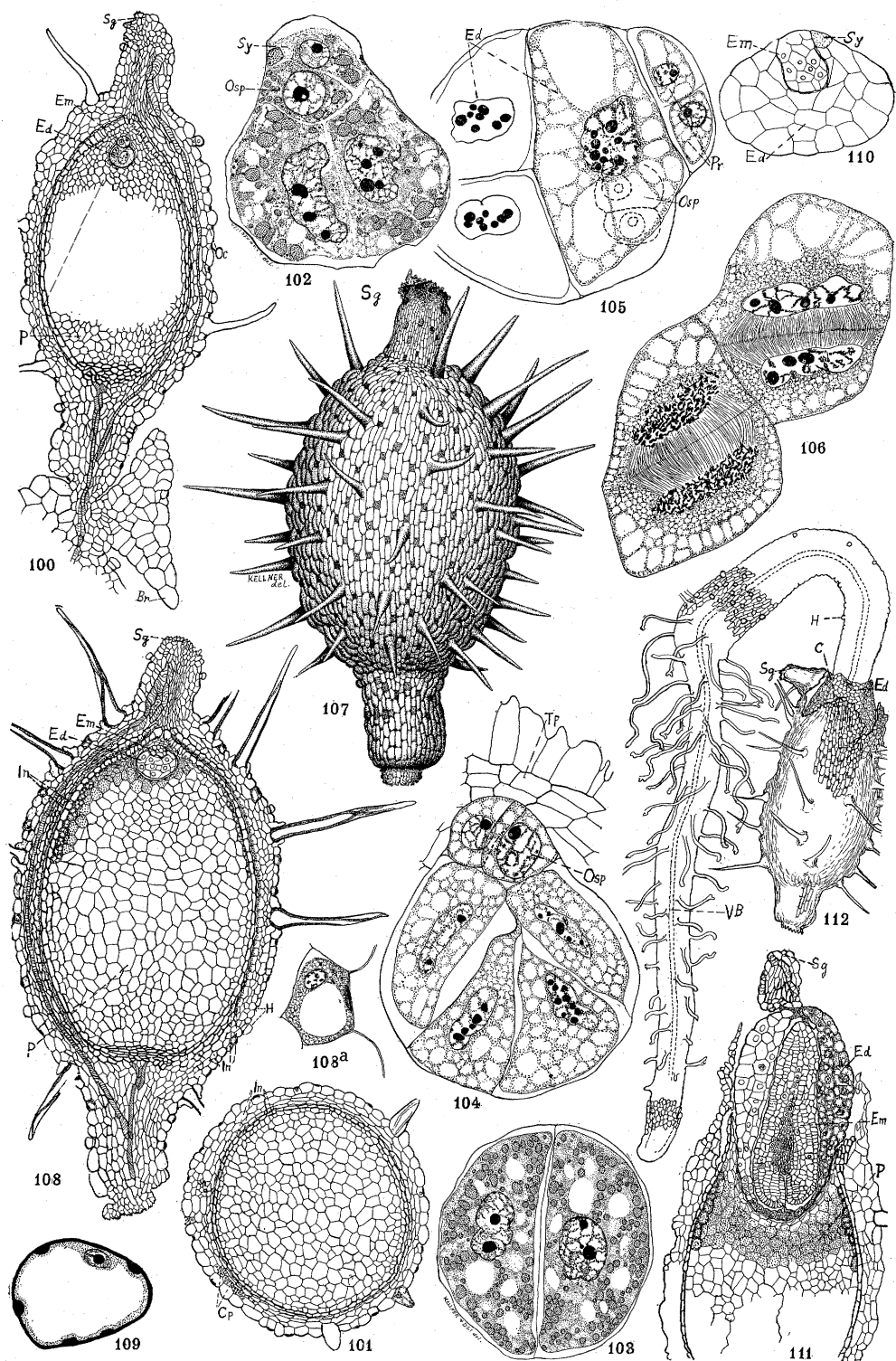
FIG. 87. Longitudinal section of sac showing advanced stage of fusion of endosperm-forming nuclei, one lobe with 4 nucleoli. This sac is unique among those seen in having, in addition to the binucleolate egg nucleus and a synergid nucleus, two additional nuclei in the micropylar lobe. They may possibly have come from the pollen tube. The number of nuclei in the fusion mass could not be counted with certainty. $\times 530$.



D. S. J. del.



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D. S. J. and Kellner del.

JOHNSON: PEPEROMIA HISPIDULA.

FIG. 88. Similar section showing the single endosperm nucleus. Fusion is complete except for the lobing that indicates the composite origin of this nucleus. The nucleoli have already fragmented to form 26 nucleoli. Oospore with one double-sized nucleolus and a large, somewhat beaded chromatin net of apparently the same character throughout. $\times 530$.

FIG. 89. Longitudinal section of an embryo sac and surrounding tissues, showing fertilized egg and one section of the single, much-lobed, endosperm nucleus. $\times 480$.

FIGS. 90-97. Successive longitudinal sections of the same endosperm nucleus that is shown in Fig. 89, showing details of the lobing and the aggregation of the chromatin along the lines of fusion of the constituent nuclei. $\times 480$.

FIG. 98. Longitudinal section of sac showing the oospore and the first mitotic spindle of the endosperm nucleus, with part of its very numerous chromosomes. $\times 530$.

FIG. 99. Similar section showing later phase of first mitosis of endosperm nucleus. The spindle has the usual, transverse position. $\times 530$.

FIG. 100. Sagittal section of a half-mature fruit and its bract, showing oospore, endosperm, perisperm, integument and carpel, with its bristles and single vascular bundle, on the abaxial side. $\times 55$.

FIG. 101. Transverse section of a somewhat older fruit, showing perisperm, integument and carpel. $\times 55$.

FIG. 102. Longitudinal section of embryo sac with oospore and 2 endosperm cells, showing the many nucleoli and the lobing of the endosperm nuclei. $\times 480$.

FIG. 103. Transverse section of similar sac showing the first 2 endosperm cells, with their lobed nuclei. Numerous fat globules in the cytoplasm. $\times 480$.

FIG. 104. Longitudinal section of sac with oospore and 4 endosperm cells. $\times 480$.

FIG. 105. Transverse section of a sac with 3 endosperm cells having lobed, multinucleolate nuclei, and 2 peripheral cells with small nuclei having a single nucleolus each. The oospore and synergid (in dotted outline) are from an overlying section. $\times 480$.

FIG. 106. Part of a longitudinal section of an embryo sac showing the numerous chromosomes of the endosperm nucleus and two stages in the cell plate that is formed at each division of the latter. $\times 1,160$.

FIG. 107. Lateral view of surface of mature fruit. $\times 55$.

FIG. 108. Sagittal section of nearly mature fruit. $\times 55$.

FIG. 108a. Part of the same section showing structure of an oil-cell. $\times 520$.

FIG. 109. Transverse section of bristle of nearly mature fruit, showing the internal, slightly spiral thickenings. $\times 480$.

FIG. 110. Longitudinal section of a mature embryo, endosperm and synergid. $\times 225$.

FIG. 111. Longitudinal section of germinating seed, showing elongated embryo still enclosed by the swollen, active endosperm. The upper part of the carpel is pushed off by the endosperm. The perisperm is partially exhausted of starch near the endosperm. $\times 55$.

FIG. 112. Surface view of young seedling with cotyledons still embedded in the endosperm. (From a glycerine mount.) $\times 25$.